

III. "Further Observations on the Gestation of Indian Rays; being Natural History Notes from H.M. Indian Marine Survey Steamer 'Investigator,' Commander R. F. Hoskyn, R.N., Commanding. Series II. No. 2." By J. WOOD-MASON, Superintendent of the Indian Museum, and Professor of Comparative Anatomy in the Medical College of Bengal, and A. ALCOCK, M.B., Surgeon, I.M.S., Surgeon-Naturalist to the Survey. Communicated by Professor M. FOSTER, Sec. R.S. Received October 9, 1891.

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§ 1. Introduction.

On the 24th of February of this year we communicated the results of some observations on the uterine villiform papillæ, or trophonemata, of *Pteroplatea micrura* (Bl. Schn.) and their relation to the embryo, and also incidentally referred to the structure and probable functions of similar uterine papillæ in *Trygon bleekeri* and *Myliobatis nieuhofii*.

We were able to show that in the pregnant females of these Batoids the mucous membrane of the uterus is extended in the form of elongate papillæ, the entire surface of which, again, is beset with tubular glands, and we were able to bring forward evidence in favour of the view—more especially in the case of *Pteroplatea micrura*—that the function of these glands is to secrete a nutritive fluid which is conveyed down the pharynx and into the stomach of the embryo.

Since the date of that communication we have been fortunate enough to obtain, in the course of the "Investigator's" survey of the Godávári Delta, pregnant and parturient females of *Trygon walga*, Müller and Henle, a pregnant female of *Myliobatis nieuhofii* (Bl. Schn.), numerous pregnant females of *Pteroplatea micrura* (Bl. Schn.), and an unimpregnated uterus of *Narcine*, the examination of which appears to corroborate the view advanced by us as to the function of the uterine papillæ and their relation to the embryo.

In the case of *Pteroplatea micrura*, of which species we have examined over a score of pregnant females, we find that the oviduct is dilated into an uterus on both sides of the body equally, and that each uterus may contain from one to three embryos, the usual number being two. In the early stages of gestation the entire surface of the

mucous membrane of the uterus is beset with trophonemata, but with the growth of the fœtus the trophonemata become atrophied by pressure, except opposite the spiracles of the fœtus, where they remain as large bunches which penetrate through the spiracles deep into the fœtal pharynx. We find that where, as appears to be the most common condition, two embryos occupy the uterus, the one is rolled up within the other—head to head and tail to tail—but in such a way as to leave the spiracles of the inner one exposed for the trophonemata to enter. In one remarkable instance, where one spiracle of the inner embryo was overlapped and concealed, that spiracle was of diminutive size, while its fellow of the opposite side was much enlarged. On microscopic examination we find the nutrient secretion of the trophonemata to contain numerous small granular bodies, and a few large granular corpuscles which resemble leucocytes.

In the pregnant female of *Myliobatis nieuhofti* we found three young ones, two males and a female, of different sizes, in an uterine enlargement which exists on the left side only. In the two smallest there are very delicate external gills; but of such structures there is no trace whatever in the largest embryo.

The spiracles in all are singularly large and patent, being kept open by an eave-like extension of the cranial cartilage. The mucous membrane of the uterus, which is thickly beset with long branched glandular villi, was intensely vascular, had an odour, not of fish, but like that of raw beef, and was covered with a creamy yellowish-coloured fluid somewhat resembling "laudable pus" in appearance. It may be mentioned that the spiral gut of one of the embryos was found to be full of the same creamy fluid, unchanged. Apparently loose in the body cavity, in the largest fœtus, were found strings of bead-like concretions held together by inspissated albuminous material: and on examination under the microscope these concretions were found to contain numerous crystals of oxalate of lime and dumb-bell-shaped bodies exactly resembling the dumb-bell concretions of urate of ammonia found in human urine.

As one of us has already described ('Journal Asiatic Society Bengal,' vol. 59, Part II, pp. 54 and 55) the general structure of the uterus and uterine glands in the stage preparatory to pregnancy in *Myliobatis nieuhofti*, we are deferring a report on this species in the hope of obtaining ampler material to make the report more complete.

We devote this paper to some account of the phenomena of gestation in *Trygon walga*, in which we have been able to make fuller observations.

§ 2. *On the Parturient Female and New-born Young of Trygon walga.*

Two females of *Trygon walga*, Müller and Henle, were taken at Cocanáda, in the Godáviri Delta, on the 8th of April of this year. The abdomen, in one of them, was so much distended that the normal flat shape of the fish was obscured. On transfer of this specimen to a bucket of sea-water, two young ones were seen to be suddenly extruded from the cloacal orifice—one a few minutes after the other. The young ones swam about vigorously in the bucket. Relatively to the size of the abdominal cavity of the mother, which in this species is much contracted, the young ones are enormous; and on seeing the two of them beside the open maternal abdomen it appears almost incredible that they could ever have been compressed into such a confined space.

The following are the measurements of the mother and offspring, with their weight after preservation in spirit:—

	Mother.	New-born young one, No. 1.	New-born young one, No. 2.
Length of disk	205 mm.	67 mm.	67 mm.
Breadth of disk	185 "	70 "	72 "
Length of tail	180 "	85 "	95 "
Length of snout from margin of fronto-nasal process to tip	57 "	19 "	19 "
Weight	2734 grains	200 grains	174 grains

Both young ones were females; neither of them had any traces of external gill filaments; both of them had a small papilliform umbilical vesicle about 1 mm. long. The spiracles in both were large and widely open.

Before going on to describe the visceral anatomy of the young, and the structure of the uterus and its trophonemata in the adult, there are certain interesting external characters, distinguishing the young from the mature female, which are important enough to be mentioned.

(1.) In the young, the dorsal surface of the body is quite smooth and devoid of the dermal tubercles which, in this situation, characterise the adult. (2.) The young have only one tail spine, whereas the adult has two, and in front of them a long series of fixed spinelets. (3.) The middle third of the space between the base of the tail spine and the tip of the tail is occupied in the young one by a median fold

of the dorsal integument 15 mm. long and about 0.75 mm. in height; this is, no doubt, a vestige of the vertical system of fins, of which no trace exists in the adult. (4.) The ventral fins in the young are placed relatively farther back than they are in the adult, projecting considerably beyond the hinder margin of the disk, while in the adult they barely reach this limit. (5.) The tail is relatively a good deal longer in the young.

The young are pigmented in all respects like the adult.

In the young one, immediately after it has left the uterus, the abdomen is very tumid, its anterior wall being stretched so thin that the abdominal and intestinal contents can be seen through it. On opening the abdomen, its cavity is found to be almost completely filled by the enormously distended colon (spiral gut), the liver, stomach, and duodenum being displaced forward beneath the pectoral girdle; the rectum is very sharply marked off from the colon, and appears as a narrow cord; the œsophagus and stomach are empty, but the colon is filled with bile-stained granular material, which, under the action of spirit, has become a hard, yellowish-brown cake, of which the weight is no less than one-sixth to one-seventh of that of the entire body.

The large relative size of the rectal gland is remarkable, not only in this species, but also, we may mention, in the foetus of *Myliobatis nieuhofti* and *Pteroplatea micrura*.

§ 3. *On the Uterus and Trophonemata of Trygon walga at the Close of Pregnancy.*

The abdomen of the mother was laid open immediately after the birth of the young ones. The right ovary and oviduct are undeveloped; the left ovary is large, and the distal end of its oviduct is dilated into a pyriform uterine swelling, the aperture of which projects into the cloaca as a conspicuous *os uteri*. On opening the uterus, which is much contracted when empty, we find a moderately thick fibrous and muscular wall, lined internally with a mucous membrane which is everywhere produced into long papillæ (trophonemata); these are brittle and friable.

The trophonemata, which are cylindrical, unbranched, and taper slightly from base to apex, are about 10 mm. in length and about 1 mm. in breadth; and, on examination with a low magnifying power, their surface is seen to be granular and much fissured.

A transverse section through the uterine wall shows, from without inwards, (1) a thin layer of fibrous tissue, (2) a layer of muscular fibres cut transversely, (3) a layer of muscular fibres cut longitudinally, and (4) the vascular submucosa and the mucosa about to be described as they appear in a trophonema.

Sections, both transverse and longitudinal, through a trophonema display a central core of fine connective tissue, in which, besides arteries, veins, and a dense capillary plexus, are cells and very numerous free leucocytes; and surrounding or external to this a series of solid finger-shaped coagula formed of confluent cells in various stages of degeneration. At the one extreme, these finger-shaped masses are seen to be made up of desquamated cells the protoplasm of which has simply become confluent into a solid mass wherein the nuclei, with the nuclear network very clear and conspicuous, stand out distinct and unchanged; while, at the other extreme, are found nothing but solid granular coagula in which neither nuclei nor structure of any kind can be distinguished, with leucocytes scattered between them.

We may anticipate events by stating that these solid masses of cells and granular coagula appear to be "epithelial casts" of the glands which, as we shall show, invest the surface of the trophonemata in the earlier stages of pregnancy, but which, at the close of pregnancy, are undergoing coagulative degeneration, while the leucocytes present appear to be exercising a resorptive function.

§ 4. *On the Uterus and Trophonemata of Trygon walga at the Beginning of Pregnancy.*

The second female specimen is about the same size as the first, but had not the same convexity of the abdomen. It also has the right ovary and oviduct undeveloped, while the left ovary is enlarged and distended with ova, and the distal end of its oviduct is dilated into a pyriform uterine chamber, which, however, is smaller than in the first specimen—measuring only 32 mm. from the fundus to the os. An egg which had recently descended into the uterus was ruptured accidentally in dissection.

The uterus has at this stage in all respects the same form as it has at the end of pregnancy; but the trophonemata, on the contrary, are as strongly contrasted as possible in the two stages. For while in the late stage they have the form of an elongated cone and are in process of disintegration, in the early stage they are strap-shaped and are in process of integration. That the trophonemata were not functioning, their incompletely evolved condition proves, apart from the consideration that in the well-filled yolk-sac there would be abundant sustenance for the embryo for some time.

The trophonemata are somewhat wavy, rather thick and fleshy, strap-shaped bodies, measuring about 10·5 mm. in length by about 1 mm. in greatest breadth. Narrow at their origin, they almost immediately widen out to their greatest breadth, which is maintained to about the seventh tenth of their length, whence they taper to their

rounded extremity. They are with difficulty straightened out from their wavy curl. In stained preparations a dark unbranched line is seen following the sinuosities of the trophonema, nearer to one margin than to the other, and tapering away to nothing at the apex; it is the rounded thickening formed by the (developing) axial vein which is so conspicuous in the trophonemata of *Pteroplatea*.

A trophonema, lightly stained with borax carmine, mounted in spirit and glycerine, and viewed under a Zeiss D ocular 2 by transmitted light, shows a darker and broader median band and two lighter and much narrower marginal bands. In the former the axial vein presents itself as a streak with a pale axis, which transverse sections prove to be the optic expression of the commencing lumen. In each of the latter a large vessel, which in transverse section is seen to be an artery, can readily be made out. By careful focussing, the surface of the broad median band is seen to be covered with a coarse polygonal network, with paler meshes, which correspond to the simple or compound duct-openings of subjacent glands; the network, like the surface of the pale marginal bands, is covered continuously with minute flat glassy cells having sharply defined nuclei and distinct limiting membranes. Some preparations suggest that, in trophonemata which are less advanced in development than the one under description, this layer of pavement cells may form a continuous investment over the whole trophonema.

Transverse sections of a trophonema show that the meshes of the polygonal network above referred to coincide with bulb-shaped nests of cells which are the still solid foundations of glands.

These glands are arranged, as in *Pteroplatea* (*vide* 'Roy. Soc. Proc.,' vol. 49, Pl. VIII, fig. 5), perpendicular to the surface, side by side, in the substance of the mucosa, but not quite so close together. They are squat bulb-shaped organs, and are often compound, with two, three, or, perhaps, four acini. In consequence of the absence of limiting cell-membranes, the exact arrangement of the gland cells cannot be made out, but the nuclei, which are elongate, are arranged somewhat irregularly in two strata—the nuclei of the two strata and of the same stratum overlapping one another; whence it may be inferred, though no cell-boundaries are traceable, that the gland tissue forms a two-layered stratified columnar epithelium similar to that which is found in many parts of the Mammalian respiratory tract.

The glands, which, as above stated, are quite solid without any trace of lumen, appear to have originated as ingrowths of the indifferent layers of the epithelium alone, the outer layer of flat glassy pavement cells which invests the surface of the trophonema between the glands and at its sides not having been involved in the process. And, from appearances presented by less developed trophonemata,

we infer that, in early stages, the pavement layer forms a continuous investment over the whole trophonema; and that subsequently, by the separation from one another at definite spots of the pavement cells, stomata are formed, which, when the lumina of the glands are established, become the mouths of the glands. Be this as it may, the outer (pavement) layer is not traceable into the mouths of the glands at any point in any of our sections.

The trophonemata possess an exceedingly rich vascular supply. All the sections show two large, but not very thick-walled, arteries, one in each non-glandular margin; minute arterioles here and there between adjacent glands immediately beneath the epithelium; and, between the layers of glands of opposite faces, a plexus of sinuous cavities or capillaries.

The capillary plexus in transverse sections of a trophonema is seen to extend deeply between the glands right up to the superficial arterioles; and, in the part corresponding to the position of the great axial vein of *Pteroplatea*, it presents a solid or spongy circular expansion, in the centre of which the future lumen of the vessel is commencing to be formed. In transverse section of a group of glands the capillary plexus is seen to form a polygonal network, in the meshes of which the glands lie.

In comparison with *Pteroplatea micrura*, the trophonemata of *Trygon walga* are characterised by the possession of an epithelium which is several cells thick instead of one cell thick, and by the richer vascular supply of their glands, each of which is embedded in a little capillary cup of its own, like a filbert in its husk.

§ 5. Conclusions.

1. Comparison of the trophonemata in the two stages above described, showing, on the one hand, at the onset of pregnancy, a mucous membrane of large nucleated indifferent cells and of solid unformed glands, and, on the other hand, when the term of pregnancy is fulfilled, a surface layer of gland-casts of epithelium in various stages of degeneration, appears to be conclusive proof that the glands are developed for the special requirements of the pregnant state.

2. As regards the function of these glands, the presence in every case where a fresh pregnant uterus has been examined of a viscid turbid or actually milky albuminous fluid, and further the finding in the case of *Myliobatis nieuhofii* of one and the same secretion in the uterus of the mother and in the intestine of the foetus, seem to fully confirm our original conclusion that they are in all cases milk-glands furnishing a secretion for the nourishment of the embryo.

3. Regarding the channel through which the milk is carried into the foetus, we think it to be in every species that we have examined,

Pteroplatea micrura, *Myliobatis nieuhofti*, *Trygon walga*, and *Trygon bleekeri*, the large wide-open spiracles. In *Pteroplatea* we know that the trophonemata pass into the spiracles; but the singular distension of these orifices in the other species, in contrast to the smallness of the other apertures of the body, points to the conclusion stated.

4. The stomach in all cases that we have hitherto observed (except in *Trygon bleekeri*, where the observation was lost) is empty, small, and displaced; while the colon (spiral gut) is full, large, and distended at the expense of other organs. And this leads us to the conclusion that the foetal stomach is simply a channel through which the easily assimilable food passes to be absorbed by the spiral gut. And of this conclusion the presence of the unchanged "milk" in the spiral gut of *Myliobatis* is corroborative.

5. Finally, as to the method of respiration of the foetus, no conclusion can as yet be arrived at. It is probably safe to assume that the consumption of oxygen and the production of carbonic acid by the foetus are comparatively small, and that the respiratory exchanges are sufficiently carried out through the soft foetal skin where this comes in contact with the vascular trophonemata and uterine wall.

Hitherto, we have not found any special distribution of blood-vessels to the skin, in the foetus. And in *Pteroplatea* the manner in which, when two foetuses are present, the one is rolled up within the other, prevents contact of the inner foetus with the uterine wall, except at the snout, and where the trophonemata enter the spiracles.

We are indebted to Professor G. B. Howes for calling our attention to a short note by Dr. W. A. Haswell ('Proceedings Linnean Society, New South Wales,' vol. 3, 1889, pp. 1713 to 1716) on *Urolophus*, in which it is suggested that the extraordinarily long external gills of the foetus are concerned in absorbing matter which is supposed to exude from the blood-vessels of the uterine villi.

IV. "On some of the Variations observed in the Rabbit's Liver under certain Physiological and Pathological Circumstances." By T. LAUDER BRUNTON, M.D., B.Sc., F.R.S., and SHERIDAN DELÉPINE, M.B., B.Sc. Received October 22, 1891.

(Abstract.)

Under the influence of the natural stimulus of digestion, numerous changes are observable in liver cells. In this we partly confirm and partly complete (and add to) the observations of previous investigators.